Immune hemolytic anemia due to diclofenac

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A 37-year-old male presented with severe anemia, mild jaundice, and hemoglobinuria during his second course of diclofenac for gout. The peripheral blood showed microspherocytes and nucleated red blood cells (RBCs). The reticulocyte count was 21 percent and haptoglobin was < 0.1 g/L. A presumptive diagnosis of diclofenac-induced immune hemolysis was made and blood, urine, and drug samples were referred for investigation. Direct antiglobulin testing showed the RBCs to be coated with IgG1, IgG4, and C3d, but an eluate only yielded weakly reacting IgG antibodies. In tests for drug-dependent antibodies, group O, R_1R_2 red cells were incubated with the patient's serum that had been mixed with either urine (which contained diclofenac metabolites) or diclofenac solution and then tested by an antiglobulin method. Strongly positive reactions with anti-IgG occurred in the tests using urine but only weak reactions in those tests employing diclofenac solution. All controls gave negative results. These findings support the role of diclofenac in causing hemolysis and the importance of employing urine as a source of drug metabolites. The findings also showed that an immune complex mechanism predominated and that the eluted IgG (detectable independently of the presence of the drug or its metabolites) confirmed a minor autoimmune component. Diclofenac was stopped and treatment with prednisolone and folic acid instituted; this resulted in complete recovery. ***Immunohematology*** 1997;13:9–11.

Drug-induced immune hemolytic anemia is a rare but potentially serious event that occurs in about 1 in 1 million of the population.[1] The subject is complex and for an in-depth consideration, one of the excellent recent review articles is recommended.[1-4] Briefly, drug-induced immune hemolysis is often divided into three main types. The drug adsorption or hapten type is usually associated with large doses of medication (e.g., penicillin, cephalosporin, or carbimazole) that coat the patient's red blood cells (RBCs). IgG-class antibodies are formed against a combination of drug- and RBC-membrane and cause the cells to be destroyed by the mononuclear phagocyte system. The second type, the immune complex type, can occur when small doses of drugs are taken, often for the second time. Antibodies are produced against the drug (examples include quinine, rifampicin, and tolbutamide) and immune complexes are formed, which then attach to the RBCs and activate complement. The resulting intravascular hemolysis may be severe and lead to renal failure or even death. In the third type, patients develop autoantibodies indistinguishable from those found in warm autoimmune hemolytic anemia. In many cases, there is little or no clinical effect, but in a few patients overt hemolysis develops. It has been postulated that the drugs (e.g., methyldopa, levodopa, or nonsteroidal anti-inflammatory agents such as ibuprofen, mefenamic acid, and naproxen) affect the T suppressor lymphocyte control of B lymphocytes, permitting the production of autoantibodies.

Individual patients may display features of more than one type of drug-induced hemolysis, and here we report a patient with severe intravascular hemolytic anemia due to diclofenac in which both immune complex and autoimmune mechanisms were implicated.

Case Report

A 37-year-old male was admitted to the hospital with symptomatic anemia. Approximately 6 weeks previously he had presented to his family doctor with gout and had been prescribed diclofenac, 50 mgs three times daily, for 4 weeks. The therapeutic response was satisfactory and the treatment was tolerated without a problem. However, within 2 weeks of stopping the drug, the gout symptoms returned and a second course of diclofenac was prescribed at the same dose. During the first week of retreatment, the patient became increasingly fatigued, mildly jaundiced, and passed dark urine. Tests for hemoglobinemia were not done. His blood counts were hemoglobin 68 g/L, white cells 13.3×10^9/L (with a neutrophilia), and platelet count 293×10^9/L. The direct antiglobulin test (DAT) was positive. A peripheral blood smear showed polychromasia, microspherocytes, and nucleated RBCs, and the reticulocyte count was 21 percent. A marrow aspiration revealed erythroid hyperplasia. Serum haptoglobin was < 0.1 g/L (normal range is 0.4–2.0 g/L) and urinalysis confirmed hemoglobinuria. A presumptive diagnosis of intravascular immune hemolytic anemia due to diclofenac was made and subsequently confirmed by investigations (involving blood, urine, and drug samples). The diclofenac was stopped and a 4-week course of prednisolone and folic acid was instituted. This

resulted in complete hematologic recovery and the patient has remained well.

Materials and Methods

The methods for immunohematologic investigations routinely carried out at the Sheffield Transfusion Centre have been described elsewhere.[5] These methods included DATs using anti-IgG, -IgA, -IgM, -C3d, -C3c, and -C4c reagents produced at our Centre. IgG subclass reagents were obtained commercially (Eurogenetics, Hampton, UK). RBC eluates were prepared by acid elution using the ELU-PLUS system (Dominion Biologicals, Dartmouth, Nova Scotia). The patient's serum was tested against a panel of nine group O cells prepared at our Centre, and an autocontrol, in saline at 18° C, and at 37° C using papain and LISS indirect antiglobulin tests (IATs) with an anti-IgG+C3c reagent. Eluates were tested against a three-cell panel by the IAT.

The tests for drug-dependent antibodies were based on previously described methods.[6,7] Briefly, 1 drop of group O, R_1R_2 RBCs (3% in saline) were incubated at 37° C with the patient's serum mixed with either 1 drop of undiluted urine (containing diclofenac metabolites) or with diclofenac solution (1 mg/mL) in phosphate-buffered saline (pH 7.0), and then tested by an antiglobulin technique using monospecific anti-human IgG and anti-human C3c. Group AB serum, urine from an individual not taking diclofenac, and phosphate buffered saline replaced the patient's serum and urine and the drug solution, respectively, as negative controls.

Tests for hemolysins were performed at 37° C using papain-treated RBCs, complement-enriched patient's serum, and an incubation time of 2 hours. For the Donath-Landsteiner test, serial dilutions of complement-enriched patient's serum were mixed with normal group O RBCs at 37° C. One set of dilutions was placed in melting ice (0° C) for 1 hour, and then incubated at 37° C for 1 hour. A second set of dilutions (control) remained at 37° C for 2 hours. A Ham's acidified serum lysis test was carried out using acidified, complement-rich sera and patient's RBCs.

Results

The patient's serum contained a weak (microscopic) panagglutinin with no apparent specificity detected at 37° C using a papain technique. The patient's blood group was O, R_1r and the DAT was strongly positive (macroscopically) with anti-IgG1, -IgG4, and -C3d. An eluate yielded a weak nonspecific IgG antibody. Tests for warm hemolysins, Donath-Landsteiner antibodies, and paroxysmal nocturnal hemoglobinuria were negative.

The results of the tests for drug-dependent antibodies are shown in Table 1. The patient's serum plus urine containing diclofenac metabolites was strongly positive versus group O, R_1R_2 RBCs in the IAT using anti-IgG. The same test using a diclofenac solution was weakly positive. All controls were negative when tested in the same manner.

Discussion

The finding (Table 1) of a strongly positive IgG reaction in the tests involving urine but only a weakly positive reaction with the diclofenac solution confirms the role of diclofenac in causing the hemolysis and also shows that an immune-complex mechanism was predominant. The presence of drug-independent IgG antibody in the red cell eluate indicates a concomitant autoimmune component.

Diclofenac is the only phenylacetate drug in the category of nonsteroidal anti-inflammatory agents. It has a unique chemical structure and is a popular treatment for arthritis, musculoskeletal pain, and acute gout. It is relatively free from side effects, the most common being gastrointestinal disturbances, including peptic ulceration. Immune mediated hemolysis associated with diclofenac therapy is exceedingly rare. Cases of severe, reversible autoimmune hemolytic anemia[8,9] (with thrombocytopenia)[8] and intravascular hemolysis of immune complex type, [10,11] which may be fatal,[10] have been reported. In one instance, the diclofenac-dependent antibodies showed relative Rh specificity and had a significant IgM component.[11] The degree of

Table 1. Results of tests for drug-dependent antibodies

	Indirect antiglobulin tests with group O, R_1R_2 red blood cells				
Reagents	Patient's serum plus urine from patient taking diclofenac	Patient's serum plus diclofenac solution	Patient's serum plus urine from patient NOT taking diclofenac	Patient's serum plus phosphate buffered saline	AB serum plus urine from patient taking diclofenac
Anti-IgG	Strongly positive	Weakly positive	Negative	Negative	Negative
Anti-C3c	Negative	Negative	Negative	Negative	Negative

anemia was usually severe, with hemoglobin levels commonly below 80 g/L.[9–12]

The involvement of different mechanisms in causing hemolysis in a single patient has been reported and may be relatively common.[1] It has been suggested that in patients with drug-induced hemolysis, such complicated responses are the rule rather than the exception.[12] The present findings (Table 1) are in keeping with this view and also confirm the importance of using urine to test for metabolite-dependent antibodies.[11,12] This has not always been the case. For example, in earlier reports when hemolysis was thought to be due to an autoimmune type response,[8,9] tests for diclofenac- or metabolite-dependent antibodies were not carried out. The patients had experienced severe intravascular hemolysis (hemoglobin levels 56 g/L and 41 g/L) with hemoglobinuria. These findings are unusual in a warm-type autoimmune hemolytic anemia and suggest that an immune complex mechanism may also have been present.

In conclusion, it is important to explore the potential etiological role of metabolites whenever a drug is suspected of causing immune-mediated hemolysis and no drug-dependent antibodies are detected by routine methods.

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